

**WHAT IS CLAIMED IS:**

1. A method for identifying a compound that modulates fungal tRNA splicing endonuclease activity, said method comprising:

- 5 (a) expressing a nucleic acid comprising a reporter gene in a cell, wherein the reporter gene comprises a tRNA intron;
- (b) contacting said cell with a member of a library of compounds; and
- (c) detecting the expression of said reporter gene, wherein a compound that modulates fungal tRNA splicing endonuclease activity is
- 10 identified if the expression of said reporter gene in the presence of a compound is altered relative to the expression of said reporter gene in the absence of said compound or in the presence of a negative control.

15 2. A method for identifying a compound that modulates fungal tRNA splicing endonuclease activity, comprising:

- (a) contacting a member of a library of compounds with a cell-free extract and a nucleic acid comprising a reporter gene, wherein said
- reporter gene comprises a tRNA intron; and
- 20 (b) detecting the expression of said reporter gene, wherein a compound that modulates fungal tRNA splicing endonuclease activity is identified if the expression of said reporter gene in the presence of a compound is altered relative to the expression of said reporter gene in the absence of said compound or in the presence of a negative
- 25 control.

3. A method for identifying a compound that modulates fungal tRNA splicing endonuclease activity, comprising:

- 30 (a) contacting a member of a library of compounds with a fungal cell containing a nucleic acid comprising a reporter gene, wherein said reporter gene comprises a tRNA intron; and
- (b) detecting the expression of said reporter gene, wherein a compound that modulates tRNA splicing endonuclease activity is identified if the expression of said reporter gene in the presence of a compound is

altered relative to the expression of said reporter gene in the absence of said compound.

4. A method of identifying a compound that inhibits or reduces fungal tRNA splicing endonuclease activity, comprising:

- (a) microinjecting a substrate of a tRNA splicing endonuclease into a fungal cell, wherein the substrate is labeled at the 5' end with a fluorophore and at the 3' end with a quencher, or the substrate is labeled at the 5' end with a quencher and at the 3' end with a fluorophore;
- (b) contacting said cell with a member of a library of compounds; and
- (c) measuring the activity of the tRNA splicing endonuclease, wherein a compound that inhibits or reduces tRNA splicing activity is identified if a fluorescent signal is less detectable in the presence of said compound relative to said signal in the absence of said compound or the presence of a negative control.

5. A method of identifying a compound that inhibits or reduces fungal tRNA splicing endonuclease activity, comprising:

- (a) transfecting a substrate of a fungal tRNA splicing endonuclease into a fungal cell, wherein the substrate is labeled at the 5' end with a fluorophore and at the 3' end with a quencher, or the substrate is labeled at the 5' end with a quencher and at the 3' end with a fluorophore;
- (b) contacting said cell with a member of a library of compounds; and
- (c) measuring the activity of the fungal tRNA splicing endonuclease, wherein a compound that inhibits or reduces tRNA splicing activity is identified if a fluorescent signal is less detectable in the presence of the compound relative to said signal in the absence of said compound or in the presence of a negative control.

6. A method of identifying a compound that inhibits or reduces fungal tRNA splicing endonuclease activity, comprising:

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- (a) contacting a fungal cell containing a substrate of a fungal tRNA splicing endonuclease with a member of a library of compounds, wherein the substrate is labeled at the 5' end with a fluorophore and at the 3' end with a quencher, or the substrate is labeled at the 5' end with a quencher and at the 3' end with a fluorophore; and
- (b) measuring the activity of said tRNA splicing endonuclease, wherein a compound that inhibits or reduces fungal tRNA splicing activity is identified if a fluorescent signal is less detectable in the presence of the compound relative to said signal in the absence of said compound or in the presence of a negative control.
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7. A method of identifying a compound that inhibits or reduces fungal tRNA splicing endonuclease activity, comprising:

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- (a) microinjecting a substrate of a fungal tRNA splicing endonuclease into a fungal cell, wherein said substrate is labeled at the 5' end with a fluorescent donor moiety and labeled at the 3' end with a fluorescent acceptor moiety, or the substrate is labeled at the 5' end with a fluorescent acceptor moiety and labeled at the 3' end with a fluorescent donor moiety;
- 20
- (b) contacting the cell with a member of a library of compounds; and
- (c) measuring the activity of said tRNA splicing endonuclease, wherein a compound that inhibits or reduces fungal tRNA splicing activity is identified if the fluorescent emission of the fluorescent acceptor moiety at the wavelength of the fluorescent donor moiety in the presence of the compound is decreased relative to said emission in the absence of the compound or in the presence of a negative control.
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8. A method of identifying a compound that inhibits or reduces fungal tRNA splicing endonuclease activity, comprising:

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- (a) transfecting a substrate of a tRNA splicing endonuclease into a fungal cell, wherein said substrate is labeled at the 5' end with a fluorescent donor moiety and labeled at the 3' end with a fluorescent acceptor moiety, or said substrate is labeled at the 5' end with a fluorescent

acceptor moiety and labeled at the 3' end with a fluorescent donor moiety;

- (b) contacting the cell with a member of a library of compounds; and
- (c) measuring the activity of the tRNA splicing endonuclease, wherein a compound that inhibits or reduces fungal tRNA splicing activity is identified if the fluorescent emission of the fluorescent acceptor moiety at the wavelength of the fluorescent donor moiety in the presence of the compound is decreased relative the emission in the absence of the compound or in the presence of a negative control.

9. A method of identifying a compound that inhibits or reduces fungal tRNA splicing endonuclease activity, comprising:

- (a) contacting a fungal cell containing substrate of a tRNA splicing endonuclease with a member of a library of compounds, wherein said substrate is labeled at the 5' end with a fluorescent donor moiety and labeled at the 3' end with a fluorescent acceptor moiety, or said substrate is labeled at the 5' end with a fluorescent acceptor moiety and labeled at the 3' end with a fluorescent donor moiety; and
- (b) measuring the activity of the tRNA splicing endonuclease, wherein a compound that inhibits or reduces tRNA splicing activity is identified if the fluorescent emission of the fluorescent acceptor moiety at the wavelength of the fluorescent donor moiety in the presence of the compound is decreased relative to said emission in the absence of the compound or in the presence of a negative control.

10. A method of identifying a compound that inhibits or reduces fungal tRNA splicing endonuclease activity, comprising:

- (a) contacting a fungal extract or a purified fungal tRNA splicing endonuclease with a substrate of a fungal tRNA splicing endonuclease and a member of a library of compounds, wherein said substrate is labeled at the 5' end with a fluorophore and labeled at the 3' end with a quencher, or said substrate is labeled at the 5' end with a quencher and labeled at the 3' end with a fluorophore; and

- (b) measuring the activity of said tRNA splicing endonuclease, wherein a compound that inhibits or reduces fungal tRNA splicing activity is identified if a fluorescent signal is less detectable in the presence of the compound relative to said signal in the absence of said compound or in the presence of a negative control.

11. A method of identifying a compound that inhibits or reduces fungal tRNA splicing endonuclease activity, comprising:

- (a) contacting a fungal extract or a purified fungal tRNA splicing endonuclease with a substrate of a fungal tRNA splicing endonuclease and a member of a library of compounds, wherein said substrate is labeled at the 5' end with a fluorescent donor moiety and labeled at the 3' end with a fluorescent acceptor moiety, or said substrate is labeled at the 5' end with a fluorescent acceptor moiety and labeled at the 3' end with a fluorescent donor moiety; and
- (b) measuring the activity of said tRNA splicing endonuclease, wherein a compound that inhibits or reduces tRNA splicing activity is identified if the fluorescent emission of the fluorescent acceptor moiety at the wavelength of the fluorescent donor moiety in the presence of said compound is decreased relative to said signal in the absence of said compound or in the presence of a negative control.

12. The method of claim 1, 2 or 3, wherein said compound inhibits fungal tRNA splicing endonuclease activity.

13. The method of claim 1, 2 or 3, wherein said compound enhances tRNA splicing endonuclease activity.

14. The method of any one of claims 1-11, wherein said method further comprises a step wherein the structure of the compound that modulates tRNA splicing endonuclease activity is determined.

15. The method of claim 1, 2 or 3, wherein said reporter gene encodes at least one member of the group consisting of firefly luciferase, renilla luciferase, click beetle

luciferase, green fluorescent protein, yellow fluorescent protein, red fluorescent protein, cyan fluorescent protein, blue fluorescent protein, beta-galactosidase, beta-glucoronidase, beta-lactamase, chloramphenicol acetyltransferase, and alkaline phosphatase.

5                   16.       The method of claim 1 or 3, wherein said cell is a yeast cell.

                  17.       The method of claim 2, 10 or 11, wherein said fungal extract is a yeast extract.

10                  18.       The method of any one of claims 1-11, wherein said compound is selected from a combinatorial library of compounds comprising peptoids; random biooligomers; diversomers such as hydantoins, benzodiazepines and dipeptides; vinylogous polypeptides; nonpeptidal peptidomimetics; oligocarbamates; peptidyl phosphonates; peptide nucleic acid libraries; antibody libraries; carbohydrate libraries; and small organic  
15       molecule libraries.

                  19.       The method of claim 18, wherein said small organic molecule libraries are libraries of benzodiazepines, isoprenoids, thiazolidinones, metathiazanones, pyrrolidines, morpholino compounds, or diazepindiones.

20                  20.       The method of claim 1 or 3, wherein said step of contacting a library of compounds with a cell is conducted in an aqueous solution comprising a buffer and a combination of salts.

25                  21.       The method of claim 20, wherein said aqueous solution approximates or mimics physiologic conditions.

                  22.       The method of claim 20, wherein said aqueous solution further comprises a detergent or a surfactant.

30                  23.       The method of claim 14, wherein said structure of the compound is determined by mass spectroscopy, NMR, vibrational spectroscopy, or X-ray crystallography.

24. The method of any one of claims 1-11, wherein said compound directly binds said fungal tRNA splicing endonuclease.

25. The method of claim 4, 5, 6, 7, 8, 9, 10 or 11, wherein said compound binds to the substrate.

26. The method of claim 1, 2 or 3, wherein said compound binds the tRNA intron.

27. The method of any one of claims 1-11, wherein said compound disrupts an interaction between the tRNA intron and the tRNA splicing endonuclease.

28. The method of any one of claims 1-11, wherein said compound disrupts an interaction between subunits of the tRNA splicing endonuclease.

29. A method of treating, preventing or ameliorating a fungal infection, or a symptom thereof, comprising the administering to a subject in need thereof an effective amount of a compound, or a pharmaceutically acceptable salt thereof, identified according to the method of claim 12.

30. The method of claim 29, wherein said fungal infection is a yeast infection.

31. A method of treating, managing, or ameliorating a fungal infection or a symptom thereof, comprising the administering to a subject in need thereof an effective amount of a compound identified according to the method of claim 4, 5, 6, 7, 8, 9, 10 or 11, or a pharmaceutically acceptable salt thereof.

32. The method of claim 31, wherein said fungal infection is a yeast infection.

33. A method of identifying a therapeutic agent for the treatment, management, or amelioration of fungal infection, or a symptom thereof, comprising:

(a) contacting a fungal cell-extract or a purified fungal tRNA splicing endonuclease with a substrate of a fungal tRNA splicing

- endonuclease and a member of a library of compounds, wherein the substrate is labeled at the 5' end with a quencher and at the 3' end with a fluorophore, or the substrate is labeled at the 5' end with a fluorophore and labeled at the 3' end with a quencher; and
- 5 (b) measuring the activity of the tRNA splicing endonuclease, wherein if under such conditions a compound that reduces the fluorescent signal relative to the fluorescent signal in the absence of said compound is detected; then
- (c) contacting the compound with a fungal cell and detecting the proliferation of said fungal cell,
- 10 wherein said compound is identified as a therapeutic agent for fungal infection if the compound reduces or inhibits the proliferation of said fungal cell.

34. The method of claim 33, further comprising the step of (d) testing said compound in an animal model for fungal infection, wherein said testing comprises administering said compound to said animal model and verifying that the compound is effective in treating, managing, or ameliorating the fungal infection in said animal model.

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35. The method of claim 4, 5, 6, 7, 8, 9, 10 or 11, wherein the substrate comprises a mature domain of a precursor tRNA.

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36. The method of claim 1, 4, 5, 7 or 8, further comprising a step wherein the cytotoxic activity of said compound is determined.

37. The method of claim 2, 3, 6, 9, 10 or 11, further comprising a step wherein the cytotoxic activity of said compound is determined.

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38. The method of claim 1, 4, 5, 7 or 8, further comprising a step wherein the cytostatic activity of said compound is determined.

39. The method of claim 2, 3, 6, 9, 10 or 11, further comprising a step wherein the cytostatic activity of said compound is determined.

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40. The method of claim 29, wherein said subject is a human.



41. The method of claim 31, wherein said subject is a human.